

Simple and rapid high performance liquid chromatographic estimation of atorvastatin and ezetimibe

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Abstract

A simple, precise, accurate and rapid HPLC method has been developed and validated for determination of ofloxacin and ornidazole simultaneously in combined dosage form. Acetonitrile and 20 mM phosphate buffer pH 3.0 adjusted with Orthophosphoric acid (30:70%v/v) was used as mobile phase. Detection was carried out at 300 nm using gatifloxacin as internal standard. Results of the analysis were validated statistically and by recovery studies. The proposed method is rugged and can be successfully used to determine the drug content of marketed formulation.

Key words: Ofloxacin, Ornidazole, Gatifloxacin, High performance Liquid Chromatography, Internal Standard.

1. Introduction

Ofloxacin (OFL) is fluoroquinolones antibacterial drug having broad spectrum of antimicrobial activity (Tripathi, 1999). Chemically 1-(1-(piperazinyl)-7-oxo-7H-pyrido {1, 2, 3,-de}-1, 4-benzoxazine-6-carboxylic acid (Budavari, 1994). It is commonly prescribed in respiratory tract infection, skin and tissue infection. It is reported in pharmacopeias such as USP and BP as an antibiotic (United State Pharmacopoeia, 2003 and British Pharmacopoeia, 2003). While, ornidazole (ORZ) is used as anti infective, used in the treatments of amoebiasis, dysentery, giardiasis, trichomoniasis (CIMS, 2006). Chemically (á-chloromethyl)-2 methyl-5- nitro-1 H-imidazole-1-ethanol,1-(3- chloro-2- hydroxyl-propyl)-2-methyl-5-nitro imidazole (Budavari, 1994). Literature survey shows that HPLC methods, Fluorimetric methods for determination of OFL & ORZ individually have been reported, including determination in urine, serum (Behl et al., 2005; Anandkumar, 2005; Chang et al., 2006).

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Intact, few methods in combination are also available (Natarajan and Raman, 2005; Kamble and Vanketchalam, 2005). But no method is reported for simultaneous estimation using Internal Standard (IS) gatifloxacin (Gati). Available methods are time consuming, tedious and expensive. The proposed method is rapid, simple, accurate, reproducible and successfully employed in routine analysis of both drugs simultaneously in tablet dosage form. Moreover, the proposed study is economical and less time consuming.

2. Materials and methods

Separation is carried out on an isocratic HPLC system (Prominence – Shimadzu), with LC20AT binary HPLC pump, LC20AT PDA detector, LC lab solution software, and RP-phenyl column (25×0.046mm I. D.; particle size 5µ). The reference standard of OFL and ORZ were obtained from Zim Laboratories Ltd; Nagpur. Tablets having combination of OFL and ORZ were purchased from local Pharmacy. Acetonitrile and water used were of HPLC grade. Orthophosphoric acid of analytical reagent grade was used.

2.1. Preparation of mobile phase and standard stock solution

The mobile phase used was a combination of acetonitrile and 20mM phosphate buffer pH 3.0 (adjusted with orthophosphoric acid), in the ratio of

30:70%v/v and is filtered through 0.45 μ m. The run time and flow rates were 10 min and 1 ml/min respectively. Detection wavelength of OFL and ORZ were set at 300nm. Standard stock solutions 1mg/ml of both, OFL and ORZ and of internal standard, Gati was prepared in mobile phase and used for estimation. For construction of calibration curve, the stock solutions were further diluted with mobile phase ranging from 10-50 μ g/ml for OFL and 25-125 μ g/ml for ORZ. Gatifloxacin solution was diluted to get the concentration of 20 μ g/ml. The diluted solutions were ultrasonicated.

2.2. Preparation of sample solutions

Twenty tablets of each brand weighed and an accurately weighed tablet powder equivalent to 10mg of OFL, was taken in volumetric flask (10ml). The powder was dissolved and make up to the mark with mobile phase. Then the solution was filtered through Whatman filter paper (no.41) and further diluted to concentration of 20 μ g/ml and 50 μ g/ml of OFL and ORZ, respectively. I.S. was added in the concentration of 20 μ g/ml.

2.3. HPLC method and chromatographic conditions

The mobile phase used Acetonitrile: 20mM phosphate buffer pH 3, filtered through 0.45 μ m is used in the ratio of 30:70%v/v was selected because it was found ideal to resolve the peaks of OFL and ORZ. Detection wavelength of OFL and ORZ were set at 300 nm.

2.4. Calibration of the assay

Aliquots of standard OFL and ORZ stock solutions were taken in 10 ml volumetric flasks and diluted up to the mark with mobile phase in such a way that the final concentrations of OFL and ORZ were in the range of 10-50 μ g/ml and 25-125 μ g/ml, respectively. 20 μ g/ml of gatifloxacin was added in each of the above solutions. The plot of peak areas verses respective concentrations of OFL and ORZ was obtained and found to be linear in the range of 10-50 μ g/ml and 25-125 μ g/ml with a co-efficient of correlation(r) 0.9994 and 0.99985 respectively. Working standard and sample solutions (n=5) were injected into the universal injector (Rheodyne) with injection volume 20 μ l. From the peak areas of OFL and ORZ the amounts of drugs in samples were computed (Table 2).

2.5. Validation of the method

The method was validated in term of linearity, accuracy, interday and intraday, reproducibility and specificity. The

limit of detection (LOD) and limit of quantitation (LOQ) were also determined. The accuracy of method was evaluated by carrying out recovery studies.

3. Results and discussion

Literature survey indicated that few methods have been reported for the combination, without using internal standard. Therefore it was thought to develop a versatile, speedy and cost effective RP-HPLC method for their simultaneous estimation in dosage forms. A mixture of Phosphate Buffer (pH adjusted to 3.0 with orthophosphoric acid) and acetonitrile (70:30%v/v) was found to be most suitable of the various combinations of solvents tried. With this solvent system, the chromatographic peaks were better defined and well resolved. The retention times of OFL and ORZ were found to be 2.875min and 7.229min, respectively, and for gatifloxacin the retention time was 3.377min. The typical chromatogram of the standard and sample solutions 20 μ g/ml are shown in fig. 1 and 2 respectively.

Analysis of tablets containing OFL and ORZ was carried out by using the above mentioned chromatographic conditions and detection was done at 300 nm. The average content of OFL and ORZ in brand-A (Euflox) was found to be 201.927 mg/tab, 504.056mg/tab respectively. For the brand-B (Ornof), the average content of OFL and ORZ was found to be 202.31mg/tab and 509.31mg/tab respectively. The results obtained by proposed method were close to the labeled claim of both the drugs. Accuracy of method was determined by calculating recovery of OFL and ORZ. The recovery study was performed by adding known amount of OFL and ORZ to the preanalysed drug solutions and then the samples were analyzed by the proposed HPLC method. The results indicated that the recovery of added OFL and ORZ was 98.74% and 99.02%, respectively ensuring that the method is accurate.

The intraday precision was determined by analyzing standard solution in the linearity range of calibration curve in triplicate on the same day, while interday precision was determined by analyzing corresponding standard solution daily for a period of one week. The result of the precision study indicates that the method is precise. Mobile phase in the ratio 30:70%v/v, could resolves the OFL, ORZ and

gatifloxacin with better resolutions. The linear regression equations are $y = 0.07701 + 1.133787x$ for OFL, and $y = -0.03431 + 0.044895x$ for ORZ. The high percentage of recovery of drugs indicates that the method is highly accurate. The content of drugs in two different samples (table 2), indicate that the proposed method is simple, rapid, precise and accurate for the estimation of OFL and ORZ in its pharmaceutical formulations.

Limit of detection and Limit of quantification were found to be $0.05\mu\text{g/ml}$, $0.15\mu\text{g/ml}$ and $0.13\mu\text{g/ml}$, $0.39\mu\text{g/ml}$ for OFL and ORZ respectively. LOD and LOQ show that the method is sensitive to OFL and ORZ.

Table 1: Validation and System Suitability Studies

Parameter	Ofloxacin	Ornidazole
Retention time (min)	2.875	7.229
Theoretical plates	4532	15193
Calibration range ($\mu\text{g/ml}$)	10-25	25-125
% Recovery	98.74	99.02
Resolution	-	22.081
Limit of detection ($\mu\text{g/ml}$)	0.05	0.13
Limit of quantification ($\mu\text{g/ml}$)	0.15	0.39
Capacity factor	1.43	1.553
Tailing Factor	1.085	1.137

Table 2: Assay

Formulations #	drug	Label claimed (mg)	% of amount found*	R.S.D.
Tablet 1 (A)	OFL	200	100.96	0.3485
	ORZ	500	100.81	0.4885
Tablet 2 (B)	OFL	200	101.15	0.5144
	ORZ	500	101.80	0.6833

* Mean of five determinations. #commercial preparation used were, brand A: Euflox-O, Lupin Pharmaceutical ltd, Brand B: Ornof, Aristo pharma.

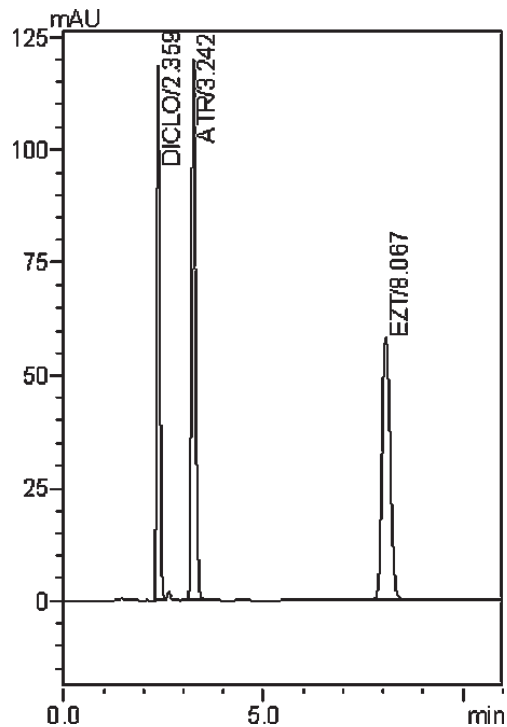


Fig: 1 Typical Chromatogram of Standard Ofloxacin, and Ornidazole, using Gatifloxacin as IS.

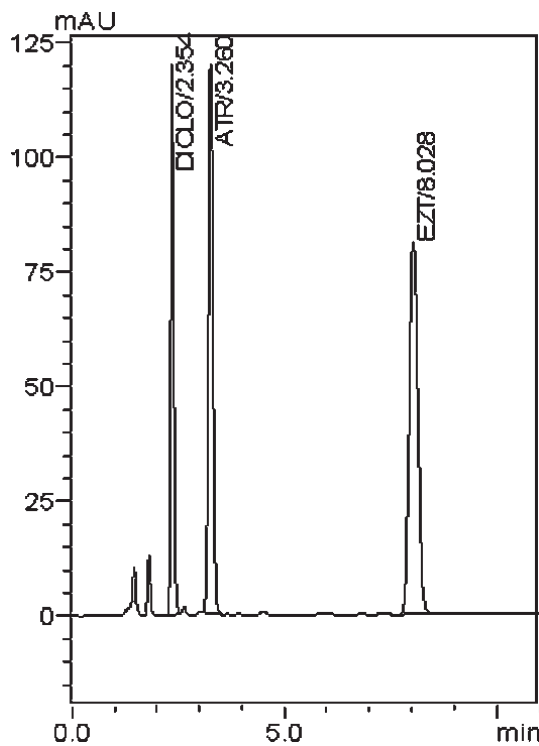


Fig: 2 Chromatogram of sample Ofloxacin, and Ornidazole, using Gatifloxacin as IS.

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